Development of a Charged Particle Microbeam for Targeted and Single Particle Subcellular Irradiation

Phase II Report

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Introduction: The overall objective of this NEER project is the development of a system for high-resolution irradiation of cells. Our goal is to produce a charged-particle beam spot of roughly 1 micron which will allow dose delivery at the subcellular level. Single (or multiple) particle irradiation will also be possible, allowing us to experimentally duplicate the radiation environments of occupational and environmental exposures. In these situations virtually no cell receives more than one hit.

To develop this technology we are taking advantage of an existing proton accelerator and charged particle microbeam, originally developed for surface analysis via proton-induced x-ray emission (PIXE). To enable biological irradiations and long-term evaluation of biological consequences we are developing a specialized endstation to replace the existing PIXE hardware. This endstation will incorporate cell visualization capabilities, the capability of monitoring single hits and a long-term visualization system (camera and software) that will permit evaluation of the route of cell death following irradiation under different conditions.

Phase II accomplishments: As described in our Phase I report, we had begun investigating the potential of a vertical beamline rather than horizontal radiation delivery. The impetus for a vertical beam comes from biologists interested in using the microbeam. Cell irradiations are much easier if the cell dish is horizontal, and, more importantly, irradiations can take longer (more cells irradiated per dish) if a small amount of medium remains on the cells.

During Phase II we fully explored the options of a vertical beam, a horizontal beam, or a third option which was to develop two simultaneous lines, one vertical and one horizontal. The existing horizontal set-up makes use of a quadrupole triplet which will generate a focused beam which can be electrostatically directed at each cell on a stationary cell dish. The advantages to this approach are threefold. First, we can make use of existing equipment as the quadrupole triplet is part of the PIXE microbeam. Second, there is the potential for a very tiny beam spot with small penumbra as a result of beam focusing (rather than beam collimation). Third, cell irradiation can be extremely rapid since the beam itself is moved across a stationary cell dish. The time consuming mechanical movements of the stage become unnecessary. Rapid throughput is essential for experiments investigating low frequency events (many cells must be irradiated) such as genetic transformation. These benefits disappear if we install a vertical beam line, requiring a 90° bending magnet for the beam, and a small spot based on collimation rather than focusing. We explored the possibility of installing a second quadrupole triplet on the vertical beamline, beyond the 90° bending magnet, however this was found to require funding significantly beyond that which is available. Thus, the only way to obtain a focused beam was to maintain the existing horizontal orientation. In the end, however, the biological advantages of a horizontal cell dish were felt to outweigh the advantages of a focused beam and we began construction of a vertical beamline during Phase II.

A 90° bending magnet compatible with installation on the existing beamline, associated power supplies and connector components were designed during Phase II. This magnet has been constructed and is currently being shipped. An additional advantage to use of the bending magnet is the ability we will have to access the doubly charged helium ion, providing the range we will need if helium rather than protons are used for cell irradiations. Helium provides a significantly higher LET (linear energy transfer) and hence more cellular damage per particle.

Experimentation with a collimation-based beam began with the purchase of three collimator sizes (25, 10 and 1 μm diameter) and coupling with a solid state detector to determine detector response as a function of particle current (number). The ease with which beam alignment could be carried out as a function of collimator length was also investigated. During Phase II we also investigated various options for cell visualization and distinction between cytoplasm and nucleus. We investigated both phase contrast and fluorescence-based approaches; the advantage of the former being that exogenous stains are unnecessary. However, after consultation with the two leading biological microprobe groups (RARAF, Columbia University, NY and Gray Laboratory in the UK), we opted for the fluorescence approach. Subcellular localization is far more reliable when fluorescent stains are used, even though these have the potential (albeit small) to affect cell behavior. Accordingly, a Zeiss transmission/fluorescence microscope was purchased and set-up during Phase II. The Zeiss brand was chosen after consultation with other users on the MIT campus.

Once the bending magnet and beamline components are installed we will carry out the final steps towards cell irradiation. The first set of experiments planned involves irradiating a human fibroblast line and examining the time course of double strand break repair via BrdU labeling. The 10(1) cell line was obtained from Dr. S. Powell (Massachusetts General Hospital) and we have carried out several experiments to determine plating conditions, plating efficiency, and cell survival following particle irradiation (on another accelerator).

During Phase II we have had considerable contact with the two leading biological microprobe groups. We made a day long visit to the RARAF facility at Columbia and have been visited by Dr. Barry Michael of the Gray Laboratory in the UK. We have been fortunate in that both groups have been very willing to share their experience in all aspects of microprobe assembly with us. We also attended and presented a paper at the 4th International Workshop on Microbeam Probes and Cellular Radiation Response in July 1999.

During Phase II, Dr. Katherine Held, consultant to our NEER grant, assembled a multi-disciplined group of scientists interested in creation and/or detection of reactive species at the subcellular level. This group has been meeting bimonthly since September 1999. Plans for follow-on funding for the biological microprobe have come out of these meetings. Also, many members of the group represent additional microbeam users and several aspects of the microbeam development have been refined or redefined as a result of their advice and interests.

A further outcome of interactions with the "Subcellular Localization Group" has been the reevaluation of our initial plans for cell localization software. During Phase II we began investigating the potential for obtaining off-the-shelf software to carry out both cellular (and subcellular) localization and long-term monitoring of cells post-irradiation. We have initiated discussions with Compix, Inc. and Compucyte, Inc. to determine if this approach will be cost effective.

Request for Phase III funding:

At this point in time we wish to request Phase III funding in the amount of $157,948.